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Ploidy levels and reproductive behaviour in invasive *Hieracium pilosella* in Patagonia

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Abstract

Within a population of invasive *Hieracium pilosella* in Chilean Patagonia we found two ploidy levels, pentaploid and hexaploid. Each ploidy level was represented by one clone. Their reproductive system was apomictic (and thus replicating the maternal genome), with a low degree of residual sexuality. It is necessary to prevent the evolution of new biotypes via hybridisation with different clones of *H. pilosella* or other *Hieracium* species introduced into Patagonia.

Keywords

Hieracium pilosella, Patagonia, ploidy levels, hybridisation

Introduction

In 2010, a paper on a *Hieracium pilosella* invasion in Patagonia (Tierra del Fuego, Argentina) was published by Cipriotti et al. (2010). Earlier, similar invasive behaviour by this species was described in Chilean Patagonia in an unpublished thesis by Cárdenas Vergara (2005). Thus, *Hieracium pilosella* has evidently invaded throughout southernmost South America. One important aspect of invasion biology is connected with species reproduction, namely, how easily a species produces progeny and how variable these progeny are. This aspect of invasion biology was not addressed in either of the two papers mentioned.

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Hieracium pilosella (syn. *Pilosella officinarum*) consists of several ploidy races (cytotypes), which combine with different reproductive modes (reviewed in Fehrer et al. 2007). Three ploidy levels (the basic chromosome number is x = 9) are common in Europe, which is the native distribution area of *H. pilosella*: tetraploids (mostly sexual,), pentaploids (mostly apomictic, rarely with sexual individuals), and hexaploids (both sexual and apomictic). Heptaploid individuals have only been found rarely, and always in mixed populations with other cytotypes; hence, it has been proposed that the heptaploids originate from cytotypes of lower ploidy via conjugation of unreduced gametes (Mráz et al. 2008). Both interspecific and intercytotype hybridisation is rather common in the whole subgenus *Pilosella* (Fehrer et al. 2007). *Hieracium pilosella* is an invasive species in other parts of the world (Tasmania, New Zealand, North America, South America; a summary is given in Fehrer et al. 2007). Its invasion has been especially studied in New Zealand; apomictic tetra-, penta-, and hexaploids occur in this secondary distribution area, but sexual plants have also been observed only occasionally (Chapman and Bicknell 2000; Houliston and Chapman 2001).

Many species of the *Hieracium* subgen. *Pilosella* are facultatively apomictic, producing predominantly progeny, which is genetically identical with their maternal parent. Nevertheless, a (usually minor) proportion of their progeny are formed by sexual process. The degree of this residual sexuality varies among species and is generally unknown, having not been studied thoroughly (for the quantification of residual sexuality in three species, see Bicknell et al. 2003; Krahulcová et al. 2004). Residual sexuality may be extremely important for the formation of new genotypes, which could serve as a substrate for natural selection.

In 2005, we acquired seeds from *Hieracium pilosella* plants (and other subgen. *Pilosella* species) collected in Chilean Patagonia. Based on the collector's information on the highly extensive populations of *H. pilosella* in this area, we presumed that this species was reproducing apomictically. Therefore, we cultivated mature plants from seeds sampled in the field, and we determined their ploidy level and reproductive mode. Our data, which are complementary to those in a recently published paper (Cipriotti et al. 2010), are important for understanding the future of the *H. pilosella* invasion in Patagonia.

Materials and methods

In the summer of 2004/2005, Ladislava Filipová collected herbarium specimens of *H. pilosella* with seeds from the following localities:

Loc. 1. Patagonia, Kampenaike, Punta Arenas, Cerro Caballo; 52°43'02"S, 70°57'46"W, alt. 30 m (4 plants).

Loc. 2. Patagonia, Kampenaike, Punta Arenas, Gali 2; 52°42'27"S, 70°59'48"W, alt. 29 m (3 plants).

Loc. 3. Patagonia, Kampenaike, N margin of Punta Arenas, Domaike, 53°7'24"S, 70°52'14"W, alt. 4 m (2 plants).

The seeds were extracted from pressed fruiting plants and were sown in 2005 into pots with sterilised garden soil. Later, the seedlings were replanted, and the mature plants were kept in outdoor beds in an experimental garden at the Institute of Botany at Průhonice, the Czech Republic. Specimens of the plants sampled in the field and of the plants cultivated from their seeds are deposited in the herbarium of the Institute of Botany, Průhonice, the Czech Republic (PRA).

Ploidy level and reproductive mode were determined using standard methods and following the procedures described by Krahulcová et al. (2004). Flow cytometry of DAPI (4' ,6-diamidino-2-phenylindole)-stained nuclei was used to determine DNA ploidy level (Suda et al. 2006), and the relative seed-set of emasculated versus open-pollinated capitula was used to determine the reproductive system (sexual versus apomictic). Potential residual sexuality (i.e., the capability of the production of sexually derived progeny) was assessed with apomictic maternal plants that were pollinated in the greenhouse by an appropriate cytotype of the same species, allowing for the origins of the progeny to be detected (Krahulcová et al. 2004). Specifically, the two detected *H. pilosella* cytotypes, pentaploid and hexaploid, were crossed with tetraploid *H. pilosella*. The viability of the pollen from the tetraploid parent was sufficient for fertilisation (pollen stainability 75% – 89%), as pollen from this parent has been used successfully in previous intercytotype crosses (Krahulcová et al. 2004). Seeds obtained from pollinated apomictic plants were analysed using the Flow Cytometric Seed Screen (FCSS) method, either in its conventional version processing seed doublets (Matzk et al. 2000), or in its modified version processing pooled samples of ten seeds (Krahulcová, Suda 2006). The origins of the progeny were inferred either from the ploidy level of the embryos as compared to the maternal ploidy level (using modified FCSS for the progeny originating from hexaploid × tetraploid crosses), or from the ploidy of the embryo and of its endosperm (using conventional FCSS for the progeny originating from pentaploid × tetraploid crosses).

The clonal (or genotypic) identity of the material from different localities was determined by comparing the isozyme phenotypes of the respective cultivated plants; a combination of four enzymes (AAT, EST, LAP, PGM) was used because this system has sufficient resolution efficiency in *Hieracium* subgen. *Pilosella* (Krahulec et al. 2004). In addition, variations in chloroplast DNA (cp-DNA) were examined in selected clones and compared with that recorded in *H. pilosella* in Europe (Fehrer et al. 2005; Krahulec et al., unpublished data). The procedure used for cp-DNA analysis (Southern blotting and minisatellite fingerprinting) and the characteristic cp-DNA haplotypes distinguished in the subgenus *Pilosella* follow Fehrer et al. (2005).

Results

A total of 57 plants were cultivated from seeds that were sampled from nine maternal plants at three localities in Patagonia. All 25 cultivated progeny plants originating from the four maternal plants at locality 1 (Materials and Methods) were pentaploid.

The other progeny plants, originating from both locality 2 (three maternal plants/11 cultivated progeny plants) and locality 3 (two maternal plants/21 cultivated progeny plants), were hexaploid. All of the progeny plants were apomictic, and their morphology was highly uniform within each cytotype: this fact implied an apomictic reproductive mode in the maternal plants collected in the field. For this reason, to detect the clonal structure among their presumably apomictic maternal parents, we chose only one progeny plant from each maternal array for isozyme analysis. Analysis showed that each cytotype was composed of only a single clone. Thus, the pentaploids and hexaploids were found to be clonally uniform. These two clones differed also in their cp-DNA haplotypes. The hexaploid clone had the main group II haplotype (namely subtype II/7), which predominates in *H. pilosella* in Europe (Fehrer et al. 2005). The haplotype detected in the pentaploid clone belonged to a main group I haplotype, namely subtype I/1.

The level of residual sexuality was low in both the pentaploid and hexaploid apomictic clones. A total of 30 progeny seeds produced by the pentaploid clone pollinated by tetraploid *H. pilosella* were analysed using the FCSS method (two seeds were analysed per sample). All of these seeds (100%) had pentaploid embryos and decaploid endosperm, corresponding to autonomous apomixis giving rise to pentaploid apomictic progeny. FCSS analysis of 190 progeny seeds showed that crossing the hexaploid clone with tetraploid *H. pilosella* also generated predominantly apomictic progeny. In the respective flow cytometric histograms (10 seeds were analysed per sample), 189 hexaploid embryos were recorded in total; a clearly detectable small peak of apomictic dodecaploid endosperm was present in all of the histograms, which again corresponds to autonomous apomixis. Only one octoploid embryo (out of 190 embryos analysed) originated from the hexaploid × tetraploid cross, likely originating from an unreduced female gamete of the hexaploid maternal plant being fertilised by a diploid male gamete of the tetraploid pollen parent. Consequently, the frequency of apomixis in hexaploid apomictic *H. pilosella* was estimated to be 99.5%.

Discussion

At all three ploidy levels that are most common in *H. pilosella*, both apomictic and sexual plants are known. Nevertheless, most of the data on chromosome number and reproductive system are based on plants from its native distribution area in Europe. The plants invading New Zealand are mostly pentaploid and apomictic, although tetraploids and hexaploids have been found there rarely (Houliston and Chapman 2001; Jenkins and Jong 1996). Apomictic reproduction is evidently advantageous especially for the colonisation of new areas. However, apomixis in *Hieracium* subgen. *Pilosella* is facultative because some degree of sexuality is still present in otherwise apomictic plants (Fehrer et al. 2007). This characteristic allows the production of some sexual progeny, provided that either another clone or another related cross-compatible species occurs together with an apomictic maternal parent. In New Zealand, sexual

plants have already been found, which supposedly originated from crosses between facultative apomicts (Chapman and Bicknell 2000; Houliston and Chapman 2001). In light of this finding, the low genetic variation and low degree of residual sexuality detected in *H. pilosella* in Tierra del Fuego decrease the chances for an analogous process in this part of its secondary distribution area. Importantly, the introduction of another *Hieracium pilosella* clone into Tierra del Fuego would be dangerous because occasional hybridisation between the different clones could result in the production of new genotypes. Also worrisome is the fact that several other *Hieracium* species with the potential to hybridise with *H. pilosella* have been introduced into this area: *H. aurantiacum, H. piloselloides* (syn. *H. praealtum*), and *H. flagellare* (a hybridogenous species originated from *H. caespitosum* and *H. pilosella*) – for references see Fehrer et al. (2007). In addition, among the herbarium specimens we received from Tierra del Fuego, *H. floribundum* (a hybridogenous species originated from *H. caespitosum* and *H. lactucella*) was also present. All of these species are known to hybridise with *H. pilosella* in Europe (Sell and West 1976), and at least some of them do so in New Zealand (Morgan Richards et al. 2004).

Species of *Hieracium* subgen. *Pilosella* are known as easily hybridising, forming both stabilised hybrids (hybridogenous species) and hybrid swarms, even between different ploidy levels (e.g., Fehrer et al. 2007; Sell and West 1976). Efficient hybridisation results in the formation of new forms (either sexual or facultatively apomictic) and increases the evolutionary potential of these species (e.g., Houliston and Chapman 2001; Morgan-Richards et al. 2004).

The following measures are recommended to prevent the rapid evolution of new biotypes of *Hieracium pilosella* (and its hybrids) in Patagonia: (i) prevent the introduction of both new clones and new cytotypes of *H. pilosella*, as well as of new species of the *Pilosella* subgenus (ii) look for possible hybrids among introduced *Hieracium* species and (iii) eradicate these hybrids from sites where they currently occur.

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